ORIGINAL ARTICLE

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Type 2 Diabetes Mellitus Is Associated With Better Bone Microarchitecture But Lower Bone Material Strength and Poorer Physical Function in Elderly Women: A Population-Based Study

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of fractures according to several studies. The underlying mechanisms remain unclear, although small case-control studies indicate poor quality of the cortical bone. We have studied a population-based sample of women aged 75 to 80 years in Gothenburg, randomly invited from the population register. Areal bone mineral density (aBMD) was measured by dual-energy X-ray absorptiometry (Hologic Discovery A), bone microarchitecture by high-resolution peripheral quantitative computed tomography (HR-pQCT; ExtremeCT from Scanco Medical AG), and reference point indentation was performed with Osteoprobe (Active Life Scientific). Women with T2DM ($n = 99$) had higher aBMD compared to controls ($n = 954$). Ultradistal tibial and radial trabecular bone volume fraction (+11% and +15%, respectively), distal cortical volumetric BMD (+1.6% and +1.7%), cortical area (+11.5% and +9.3%), and failure load (+7.7% and +12.9%) were higher in diabetics than in controls. Cortical porosity was lower (mean ± SD: 1.5% ± 1.1% versus 2.0% ± 1.7%, $p = 0.001$) in T2DM in the distal radius but not in the ultradistal radius or the tibia. Adjustment for covariates (age, body mass index, glucocorticoid treatment, smoking, physical activity, calcium intake, bone-active drugs) eliminated the differences in aBMD but not in HR-pQCT bone variables. However, bone material strength index (BMSi) by reference point indentation was lower in T2DM (74.6 ± 7.6 versus 78.2 ± 7.5, $p < 0.01$), also after adjustment, and women with T2DM performed clearly worse in measures of physical function (one leg standing: −26%, 30-s chair-stand test: −7%, timed up and go: +12%, walking speed: +8%; $p < 0.05$-0.001) compared to controls. In conclusion, we observed a more favorable bone microarchitecture but no difference in adjusted aBMD in elderly women with T2DM in the population compared to nondiabetics. Reduced BMSi and impaired physical function may explain the increased fracture risk in T2DM. © 2016 American Society for Bone and Mineral Research.

KEY WORDS: TYPE 2 DIABETES MELLITUS; OSTEOPOROSIS; BONE MICROARCHITECTURE; BONE MATERIAL STRENGTH; BONE MICROINDENTATION; HR-PQCT

Introduction

Epidemiologic studies have shown that type 2 diabetes mellitus (T2DM) is associated with an increased risk of osteoporotic fractures,[1,2] especially hip fractures according to a meta-analysis.[3] The increased fracture risk in T2DM may seem paradoxical, because areal bone mineral density (aBMD) is higher in subjects with T2DM than in controls in many studies.[4] In general, subjects with T2DM have a higher than normal body weight and body mass index (BMI) but after adjustment for BMI, aBMD has also been reported as increased compared to healthy subjects.[5] Furthermore, at a given aBMD, subjects with T2DM have a higher risk of fracture than the normal population.[6] This has led to the conclusion that other factors, both skeletal and nonskeletal, contribute to the increased risk of fracture in T2DM.[7–9]

In addition to low aBMD, deteriorated bone microarchitecture has for long been considered to contribute to the increased risk of fractures in osteoporosis.[10] In a few relatively small case-control studies, cortical bone microarchitecture has been studied in patients with T2DM. Some studies have shown no difference in microarchitecture,[11,12] whereas others have found that T2DM with fractures compared to T2DM without fractures[13,14] or T2DM compared to controls[15–17] had worse cortical bone microstructure. Furthermore, bone material strength index (BMSi) measured by reference point indentation

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in vivo of the cortical bone in the tibia has been reported as reduced in T2DM.[12,18]

It has been suggested that advanced glycosylation end-products (AGEs) and microvascular complications could interfere with normal bone metabolism and contribute to poorer bone quality in T2DM.[18,19] In addition, nonskeletal factors such as diabetic complications, physical disability, and increased risk of falls have been suggested to contribute to the increased risk of fractures in T2DM.[20–22]

Previous studies characterizing the skeletal phenotype in T2DM have mainly included small groups of patients with diabetes attending tertiary clinics, and the control groups have in most studies been equally small, not allowing for adjustment for confounding factors known to influence BMD and bone strength.[11–18] We have performed a population-based study on women between 75 and 80 years of age with and without T2DM with regard to aBMD, bone microarchitecture, BMSi, and physical function, in order to further characterize the bone phenotype in T2DM.

Subjects and Methods

Subjects

This study is a cross-sectional study on an initial sample of 1057 women from a prospective population-based study that was performed in the greater Gothenburg area and included a total of 3030 ambulant women aged 75 to 80 years, randomly recruited via the Swedish national population register between the years 2013 to 2016. The subjects first received an invitation letter, and were then contacted by telephone. Those who accepted and were eligible to participate (were ambulant and able to follow instructions in Swedish, and had at least one hip that could be evaluated for aBMD) were scheduled for a visit (42.7%). All examinations took place at the Osteoporosis Clinic, Department of Geriatrics, Sahlgrenska University Hospital, Mölndal, Sweden.

Among the 1057 women in the present study, four had type 1 diabetes mellitus and were excluded from all analyses. A total of 74 women had known T2DM and received pharmacological treatment with metformin ($n = 61$), insulin ($n = 18$), repaglinide ($n = 6$), sulfonylureas ($n = 6$), sitagliptin ($n = 2$), exenatide ($n = 1$), and pioglitazone ($n = 1$). Of these 74 women a total of 53 had monotherapy for T2DM, 40 with metformin and nine with insulin. Twenty-five women were newly diagnosed with T2DM, and either had fasting plasma glucose above 7 mmol/L or nonfasting plasma glucose above 11 mmol/L, resulting in a group of 99 women with T2DM and 954 control subjects. The study protocol was approved by the ethical review board at the University of Gothenburg and all subjects signed an informed consent prior to participation.

Anthropometrics and physical function tests

Body height and weight were measured twice using standardized equipment and the mean values were used in the analyses. If there was more than 5 mm difference between the height measurements, a third measure was obtained and the two most similar values were used. BMI was calculated by dividing weight in kilograms with the squared height measurement in meters.

Physical function was evaluated by several tests. Timed Up and Go (TUG) is a combined measure of mobility and balance[23] and was performed by asking the women to rise from a sitting position, walk 3 m, turn around, walk back, and sit down again. The number of seconds to complete the test was recorded. In the 30-s chair-stand test, the women were asked to rise from the chair with their arms crossed over their chest and sit down again as many times as possible during 30 s. This test is a measure of lower body strength in older adults[24] and the number of complete movements from starting to rise to getting back to the sitting position was assessed. One leg standing test is a clinical balance test[25] and was performed twice for both legs after trying the test position once. The test position was to look straight ahead, have the arms crossed over the chest, and lift the lower leg back by bending the knee. The maximum time allowed was 30 s, the women were allowed to choose which leg to start with, and the highest value was used in the analyses. Grip strength was measured with a Saehan hydraulic hand dynamometer (model SH5001; Saehan Corporation, Masan, Korea) with two attempts for each hand while the lower arm rested on a flat surface and the elbow was at a 90-degree angle. An average for the dominant hand was used in the analyses. For the 10-m walk test, the women were asked to walk 10 m twice at a pace of their own choice. In order to eliminate the effects of acceleration and deceleration, the middle 6 m was timed and average walking velocity in m/s was calculated and used in the analyses.

Questionnaires

Information on medical history, medication and life-style factors influencing the risk for osteoporosis and fractures was obtained by a standardized questionnaire that was filled out by all women. Physical Activity Scale for the Elderly (PASE) was used to estimate the physical activity the last 7 days prior to assessment, and a score was calculated from participation and the number of hours per week spent in each activity multiplied by given weights and summarized.[26] Daily calcium intake was estimated by combining the amount of calcium provided by supplements with food-derived calcium intake estimated by a validated questionnaire.[27]

Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DXA) was used for assessment of aBMD and body composition. The same device was used for all subjects (Hologic Discovery A; Hologic, Waltham, MA, USA) and the BMD measurements were performed at the femoral neck, total hip and lumbar spine (L_1–L_4). A total body scan was used to assess the amount of fat and lean body mass. The coefficient of variation (CV) for women in this age group at our unit was 1.3% for the femoral neck, 0.8% for the total hip, and 0.7% for the lumbar spine.

HR-pQCT

High-resolution peripheral quantitative computed tomography (HR-pQCT) of the tibia and radius of the nondominant body side was performed using XtremeCT (Scanco Medical AG, Brüttisellen, Switzerland) in accordance with a previously described protocol.[28] All participants were measured at the standard site with the first image 9.5 and 22.5 mm from a line placed at the articular plateau in the radius and tibia, respectively (Fig. 1A). A more proximal section located at 14% of the bone length was also measured (Fig. 2A), providing a site with mainly cortical bone. A total of 110 cross-sectional images were obtained at both the standard and the 14% site as described.[29] Quality of the images was estimated in accordance with the protocol from

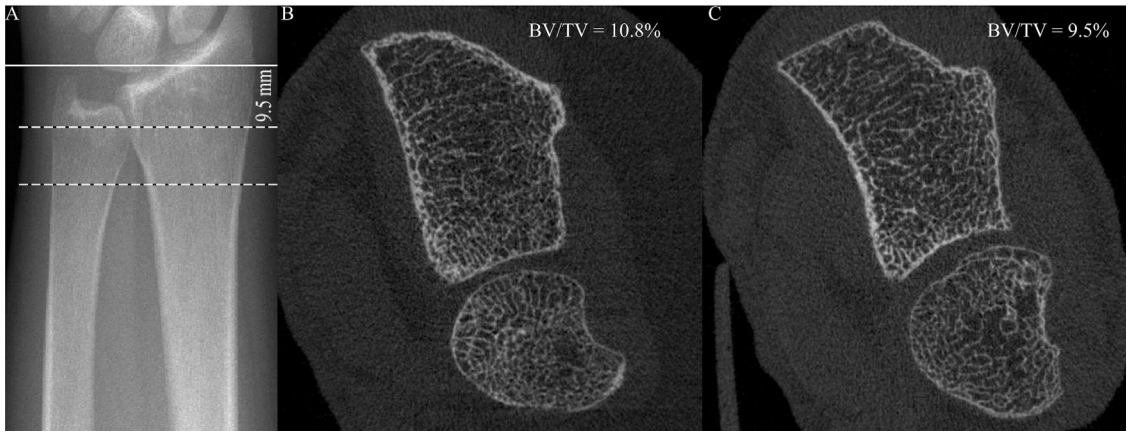

Fig. 1. Representative HR-pQCT images of the radius at the standard site, 9.5 mm from the articular plateau (*A* = scout radiograph) from a woman with T2DM (*B*) and from a control subject (*C*). BV/TV = trabecular bone volume fraction.

the manufacturers[30] and graded on a scale from 1 (best) to 5 (worst). Only scans with adequate quality (1 to 3) were used in the analyses. Volumetric bone mineral density (vBMD) and bone microarchitecture estimates were assessed from both the standard and the more proximal 14% site. CVs were calculated from measurements of six women between 75 and 80 years of age who were scanned twice at our unit.

Trabecular variables (trabecular bone volume fraction [BV/TV], number [Tb.N], thickness [Tb.Th], and separation [Tb.Th]) were analyzed as described[31,32] at the standard site where trabecular bone is abundant (Fig. 1). The CVs of the trabecular variables in the tibia were 0.8% for BV/TV, 1.9% for Tb.N, 2.6% for Tb.Th, and 2.1% for Tb.Sp.

Cortical variables were analyzed at both the standard and the 14% site using Image Processing Language (IPL v5.08b) provided by the manufacturer (Scanco Medical AG) to further process all images.[33] To delineate the bone from extra-osteal soft tissue and to separate cortical from trabecular bone, contours were automatically placed at the periosteal and endosteal sides of the cortical bone. These contours were manually corrected after careful inspection, if needed. Within the two contours, cortical vBMD and microarchitecture were assessed. Cortical bone volume (Ct.BV), cortical vBMD, and cortical area were measured, and cortical porosity was calculated as Ct.Po.V/(Ct.Po.V + Ct.BV).[33,34] The CV for cortical porosity in the tibia was 0.9% at the standard site and 4.1% at the 14% site. CVs for measurement of cortical vBMD and cortical area were 0.3% and 0.7%, respectively, at the 14% site.

Finite element (FE) analysis (FEA) was performed with models created with software from Scanco Medical AG (version V5.11/FE-V01.15), by converting the voxels to brick elements of the same size. A simulated uniaxial compression was applied and failure load was estimated as the load at which at least 2% of the bone elements surpassed 7000 microstrain.[35,36] Stiffness, the resistance against deformation, was also reported. A Young's modulus of 10 GPa and a Poisson ratio of 0.3 were used in the FE models for all subjects, in accordance with a previously described method.[35] The CVs for these measurements ranged from 0.5% to 1.5% in the radius and 0.6% to 1.2% in the tibia.

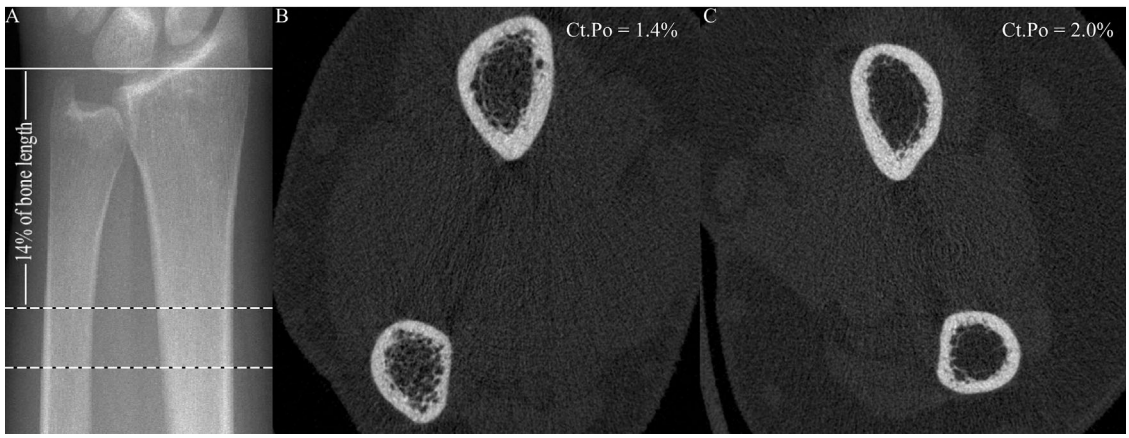

Fig. 2. Representative HR-pQCT images of the radius at the site 14% of the bone length from the articular plateau (*A* = scout radiograph) from a woman with T2DM (*B*) and from a control subject (*C*). Ct.Po = cortical porosity.

Bone microindentation

A total of 477 women (45.1%) also accepted to undergo bone microindentation.

Reference point indentation was performed with an Osteoprobe device (Active Life Scientific, Santa Barbara, CA, USA) in accordance with the instructions from the manufacturer and as described[29,37] in order to obtain estimates of BMSi. In brief, after local anesthesia, the needle of the Osteoprobe was inserted through the skin and periosteum of the anterior mid-tibia. A preload force of up to 10 N was applied to establish the probe and an impact force of 30 N was released by a trigger mechanism. The distance the probe moved in the cortical bone from the established position after release of the impact force, the indentation distance increase, was related to the distance the probe moved in a polymethylmethacrylate plastic calibration phantom and multiplied by 100 in order to obtain the BMSi for the individual patient. At least 10 valid microindentations were related to the mean of five indentations in the polymethylmethacrylate phantom for each study subject. The CV for BMSi in elderly women at our unit was 3.2%.

Vertebral fracture assessment

Vertebral fracture assessment (VFA) was performed using lateral scans by DXA and the semiquantitative classification of Genant.[38] One physician (LJ) evaluated all scans and graded the fractures as mild, moderate, or severe according to the height reduction of the vertebrae. The reproducibility was tested as previously reported[39] and the intraobserver agreement was 98.9% (kappa score 0.72) for all fractures and 100% (kappa score 1.0) for moderate and severe fractures.

Statistical analyses

Statistical comparison between subjects with and without T2DM was done with independent *t* tests for continuous variables and with χ^2 for categorical variables. Multivariable linear regression was used to adjust bone variables for covariates (age, BMI, previous and present glucocorticoid treatment, smoking, physical activity assessed by PASE, daily calcium intake, and bone-specific medication). Values are presented as mean ± SDs unless otherwise stated and *p* values less than 0.05 were considered significant. All statistical analyses were performed with SPSS Statistics Version 21 (IBM Corporation, Armonk, NY, USA).

Results

The characteristics of the women with and without T2DM in the population-based sample are presented in Table 1. The women with T2DM had a higher body weight (+13%) and BMI (+14%) than the controls ($p < 0.001$ for both comparisons). Both fat and lean body mass was higher in T2DM (Table 1). There was a tendency toward lower current physical activity as measured by

Table 1. Characteristics of Elderly Women With and Without T2DM

Characteristics	T2DM ($n = 99$)	Controls ($n = 954$)	p[a]
Age (years)	77.6 ± 1.5	77.7 ± 1.5	0.601
Height (cm)	161.8 ± 5.4	162.0 ± 5.9	0.724
Weight (kg)	76.7 ± 1 2.1	67.7 ± 11.6	0.000
Body mass index (kg/m^2)	29.3 ± 4.6	25.8 ± 4.2	0.000
Fat body mass (kg)	31.2 ± 7.2	26.5 ± 7.4	0.000
Lean body mass (kg)	45.7 ± 5.9	41.6 ± 5.3	0.000
PASE score	98 ± 55	108 ± 53	0.072
Calcium intake (mg/day)	668 ± 371	710 ± 396	0.314
Self-reported falls during the last 12 months, *n* (%)	28 (28)	298 (31)	0.545
Pharmacological treatment, *n* (%)			
Bone-active drugs[b]	3 (3)	59 (6)	0.204
Glucocorticoids, current use	3 (3)	27 (3)	0.909
Glucorticoids, previous use	18 (18)	128 (13)	0.195
Medical history, *n* (%)			
Hypertension	75 (76)	465 (49)	0.000
Stroke	10 (10)	78 (8)	0.510
Myocardial infarction	8 (8)	38 (4)	0.058
Angina pectoris	10 (10)	53 (6)	0.071
Smoking, *n* (%)			
Current	9 (9)	60 (6)	0.284
Previous	36 (36)	331 (35)	0.740
Fractures, *n* (%)			
After 50 years of age	36 (36)	359 (38)	0.804
Vertebral[c]	30 (32)	245 (26)	0.278
Vertebral or after 50 years of age[c]	52 (55)	480 (52)	0.612

Values are means ± SD unless otherwise stated.

T2DM = type 2 diabetes mellitus; PASE = physical activity scale for the elderly.

[a]Statistical comparison between T2DM and controls was made by independent samples *t* tests for continuous variables and χ^2 tests for categorical variables.

[b]Bisphosphonates and denosumab.

[c]Information is lacking for four subjects with T2DM and 26 controls.

PASE in the women with T2DM than in controls (98 ± 55 versus 108 ± 53, $p = 0.072$). More women with T2DM had been diagnosed with hypertension (76% versus 49%, $p < 0.001$) and there was no difference between the groups in the number of women who were treated with bone-active drugs (in total: oral bisphosphonates, $n = 60$; intravenous bisphosphonates, $n = 1$; denosumab, $n = 1$) or glucocorticoids or who were current or previous smokers. Three percent of the women with T2DM were treated with bone-active drugs and 6% of the controls (not significant). There was no difference in the self-reported number of falls during the last year, vertebral fractures according to VFA, or fractures after 50 years of age (Table 1). Mean duration of T2DM was 10.8 ± 6.2 years (median, 10 years; range, 2 to 25 years).

aBMD

aBMD measured by DXA of the femoral neck, total hip, and lumbar spine (L_1–L_4) was 4.5% to 8.6% higher in women with T2DM than in controls (Table 2). However, after adjustment for covariates the differences in aBMD between the groups were not statistically significant (Table 2).

Bone microarchitecture and vBMD

The trabecular bone volume fraction of both radius and tibia was significantly higher in subjects with T2DM compared to controls (+15%, and +11%, respectively, $p < 0.001$ for both comparisons), also after adjustment for covariates (Table 2, Fig. 3A). Cortical porosity of the radius was higher in subjects with T2DM at the standard site (+16%, $p < 0.01$), but lower at the 14% site (–25%, $p \leq 0.001$) whereas there were no differences in cortical porosity in the tibia at either site, neither before nor after adjustment for covariates (Table 3). There was a close correlation between trabecular BV/TV and cortical porosity at the standard site both at the radius ($r = 0.28, p = 0.000$) and at the tibia ($r = 0.19, p = 0.000$). The correlation between trabecular BV/TV and cortical porosity was clearly lower at the more proximal 14% site ($r = 0.07$, $p = 0.049$ at 14% of the radius; $r = 0.07$, $p = 0.046$ at 14% of the tibia). Adjustment for the variation in trabecular BV/TV eliminated the difference in cortical porosity at the standard site of the radius (adjusted values 4.0% ± 2.0% for T2DM and 3.7% ± 1.6% for controls, $p = 0.14$). Representative images from the standard and the 14% site of subjects with and without T2DM are shown in Figs. 1B-C and 2B-C. Also the other trabecular and cortical variables indicated better microarchitecture in subjects with T2DM than in controls both before and after adjustment for covariates (Tables 2-3, Figs. 3B-D and 4A-B).

Bone material strength and FE analysis

BMSi was clearly lower in subjects with T2DM compared to controls (74.6 ± 7.6 versus 78.2 ± 7.5, $p \leq 0.001$) also after adjustment for covariates (Fig. 5). Stiffness and failure load were significantly higher in the subjects with T2DM than in controls at both sites of the radius both before and after adjustment for covariates. At the standard site of the tibia, women with T2DM had significantly higher stiffness and failure load before, but not after, adjustment (Table 3). However, the measurement at the 14% site resulted in significantly higher stiffness and failure load in the tibia also after adjustment (Table 3). Further adjustment, also for BMSi by linear regression, attenuated the differences in stiffness (T2DM 71 ± 9 kN/mm versus controls 68 ± 10 kN/mm, $p = 0.13$) and failure load (T2DM 3521 ± 445 N versus controls 3416 ± 521 N, $p = 0.24$) at the 14% site of the radius but did not significantly alter any other results (data not shown).

Tests of physical function

The women with T2DM performed worse than controls in most physical function tests, including the one-leg standing test

Table 2. Areal BMD, Trabecular Microarchitecture, and Bone Material Strength in Elderly Women With and Without T2DM

	T2DM		Controls			
Bone variables	*n*		*n*		Unadjusted *p*	Adjusted *p*
Regional BMD (DXA)						
Femoral neck (g/cm^2)	99	0.69 ± 0.10	953	0.66 ± 0.10	0.002	0.357
Total hip (g/cm^2)	99	0.85 ± 0.12	953	0.79 ± 0.11	0.000	0.073
Lumbar spine L_1–L_4 (g/cm^2)	99	1.01 ± 0.18	948	0.93 ± 0.16	0.000	0.150
Radius standard site measurement by HR-pQCT						
Trabecular BV/TV (%)	83	11.4 ± 3.3	779	9.9 ± 3.4	0.000	0.015
Trabecular number (1/mm)	83	1.93 ± 0.37	779	1.69 ± 0.44	0.000	0.002
Trabecular thickness (mm)	83	0.058 ± 0.010	779	0.058 ± 0.011	0.846	0.917
Trabecular separation (mm)	83	0.49 ± 0.16	779	0.59 ± 0.26	0.000	0.002
Tibia standard site measurements by HR-pQCT						
Trabecular BV/TV (%)	94	13.4 ± 2.7	912	12.1 ± 2.9	0.000	0.008
Trabecular number (1/mm)	94	1.96 ± 0.35	912	1.77 ± 0.35	0.000	0.028
Trabecular thickness (mm)	94	0.069 ± 0.012	912	0.069 ± 0.013	0.863	0.281
Trabecular separation (mm)	94	0.46 ± 0.10	912	0.53 ± 0.16	0.000	0.110
Microindentation						
Bone material strength index	51	74.6 ± 7.6	438	78.2 ± 7.5	0.001	0.019

Values are means ± SD. Unadjusted *p* values were obtained by statistical comparison between T2DM and controls by independent samples *t* tests. Adjusted *p* values were from comparison of values obtained by linear regression adjusting for age, body mass index, previous and present glucocorticoid treatment, current and previous smoking, physical activity according to physical activity scale for the elderly (PASE), daily calcium intake, and bone-specific medication (bisphosphonates and denosumab).

BMD = bone mineral density; T2DM = type 2 diabetes mellitus; DXA = dual-energy X-ray absorptiometry; HR-pQCT = high-resolution peripheral quantitative computed tomography; BV/TV = bone volume fraction.

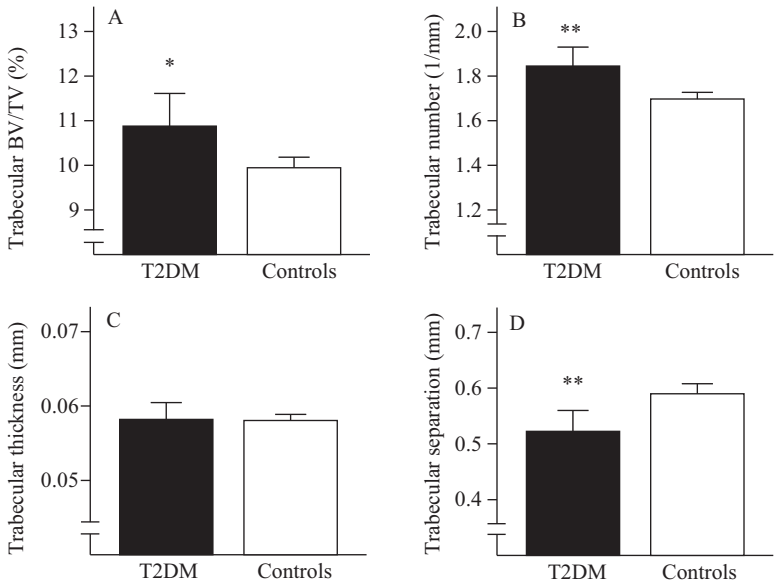

Fig. 3. Trabecular bone volume fraction (*A*), trabecular number (*B*), trabecular thickness (*C*), and trabecular separation (*D*) measured by HR-pQCT at standard site of the radius in subjects with (filled bars) and without (open bars) T2DM. Bars represent mean ± SE after adjustment for age, body mass index, smoking, current and previous glucocorticoid treatment, use of bone-active drugs, physical activity, and calcium intake. $^{*}p < 0.05$, $^{**}p < 0.01$ for statistical difference between T2DM and controls.

(13.4 ± 8.8 s versus 17.7 ± 10.4 s, $p < 0.001$), chair-stand test (10.4 ± 2.7 complete sets versus 11.2 ± 3.4 complete sets, $p < 0.05$), walking speed (1.2 ± 0.3 m/s versus 1.3 ± 0.2 m/s, $p < 0.001$), and TUG (9.6 ± 3.0 s versus 8.6 ± 3.1 s, $p < 0.01$) (Fig. 6A-D). Grip strength in the dominant arm was not significantly different in women with T2DM compared to controls (12.4 ± 5.9 kg versus 13.4 ± 5.3 kg, $p = 0.086$).

Discussion

In our study, the diagnosis of T2DM in ambulant elderly women from the population was associated with better bone microarchitecture but worse physical function and reduced bone material strength. This is of interest as there are numerous reports on higher risk for fractures in T2DM,[1–3,6] and because this is the largest cohort of T2DM with the most comprehensive bone phenotype characterization by HR-pQCT and microindentation so far in the literature.

Some of the previous smaller studies,[15–17] but not all,[11,12] have described increased cortical porosity in T2DM and the main hypothesis has been that the increased fracture rate in T2DM is mainly due to impaired cortical bone quality. Cortical porosity has previously been shown to be associated with prevalent fractures[40] and correlated to bone strength.[41] Our finding of a higher cortical porosity at the standard site of the radius in T2DM could be explained by higher trabecular bone volume fraction in T2DM, causing a misplacement of the endosteal contour. The cortical evaluation is more reliable at a more proximal measuring site, as the risk of misplacing the endosteal contour in the analysis is lower, especially in elderly subjects who have thin

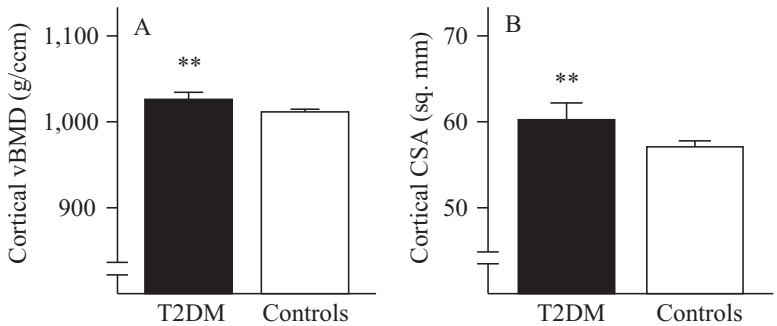

Fig. 4. Cortical volumetric BMD (*A*) and cortical area (*B*) measured by HR-pQCT of the radius at 14% of bone length in subjects with (filled bars) and without (open bars) T2DM. Bars represent mean ± SE after adjustment for age, body mass index, smoking, current and previous glucocorticoid treatment, use of bone-active drugs, physical activity, and calcium intake. $^{**}p < 0.01$ for statistical difference between T2DM and controls.

Table 3. Total BMD, Cortical Bone Microarchitecture, and Finite Element Analysis by HR-pQCT in Elderly Women With and Without T2DM

Bone variables	T2DM		Controls		Unadjusted *p*	Adjusted *p*
	n		*n*			
Distal radius measurements by HR-pQCT						
Total BMD, standard site (mg/cm^3)	76	268 ± 57	759	238 ± 62	0.000	0.008
Total BMD, 14% site (mg/cm^3)	74	584 ± 93	725	530 ± 104	0.000	0.001
Cortical porosity, standard site (%)	68	4.3 ± 2.0	625	3.7 ± 1.6	0.007	0.036
Cortical porosity, 14% site (%)	75	1.5 ± 1.1	690	2.0 ± 1.7	0.001	0.018
Cortical volumetric BMD, standard site (mg/cm^3)	68	823 ± 63	625	806 ± 63	0.029	0.178
Cortical volumetric BMD, 14% site (mg/cm^3)	75	1029 ± 35	690	1011 ± 43	0.000	0.003
Cortical area, standard site (mm^2)	68	45.4 ± 9.2	625	40.6 ± 8.6	0.000	0.013
Cortical area, 14% site (mm^2)	75	62.2 ± 8.9	690	56.9 ± 9.6	0.000	0.005
Stiffness, standard site (kN/mm)	67	61 ± 13	614	54 ± 12	0.000	0.004
Stiffness, 14% site (kN/mm)	74	74 ± 10	680	68 ± 11	0.000	0.011
Failure load, standard site (N)	67	3125 ± 662	614	2767 ± 599	0.000	0.004
Failure load, 14% site (N)	74	3676 ± 513	672	3395 ± 560	0.000	0.021
Distal tibia measurements by HR-pQCT						
Total BMD, standard site (mg/cm^3)	94	248 ± 49	912	223 ± 47	0.000	0.002
Total BMD, 14% site (mg/cm^3)	89	423 ± 70	905	381 ± 77	0.000	0.000
Cortical porosity, standard site (%)	78	11.0 ± 3.2	728	10.4 ± 2.8	0.153	0.112
Cortical porosity, 14% site (%)	75	4.4 ± 1.9	740	4.7 ± 2.2	0.224	0.255
Cortical volumetric BMD, standard site (mg/cm^3)	78	760 ± 69	728	746 ± 60	0.086	0.341
Cortical volumetric BMD, 14% site (mg/cm^3)	75	952 ± 42	740	937 ± 44	0.007	0.011
Cortical area, standard site (mm^2)	78	93 ± 19	728	83 ± 18	0.000	0.018
Cortical area, 14% site (mm^2)	75	146 ± 23	740	131 ± 25	0.000	0.005
Stiffness, standard site (kN/mm)	77	175 ± 29	710	163 ± 28	0.000	0.078
Stiffness, 14% site (kN/mm)	73	194 ± 26	727	179 ± 27	0.000	0.025
Failure load, standard site (N)	77	8921 ± 1385	710	8283 ± 1366	0.000	0.086
Failure load, 14% site (N)	73	9626 ± 1289	727	8969 ± 1199	0.000	0.041

Values are means ± SD. Unadjusted *p* values were obtained by statistical comparison between T2DM and controls by independent samples t tests. Adjusted *p* values were from comparison of values obtained by linear regression adjusting for age, body mass index, previous and present glucocorticoid treatment, current and previous smoking, physical activity according to physical activity scale for the elderly (PASE), daily calcium intake, and bone-specific medication (bisphosphonates and denosumab).

BMD = bone mineral density; HR-pQCT = high-resolution peripheral quantitative computed tomography; T2DM = type 2 diabetes mellitus.

cortices.[42] Because of the relatively high positive intercorrelation at the standard site between trabecular bone volume fraction and cortical porosity, we speculate that the estimate of cortical porosity can be compromised in areas with thin cortices and high bone volume fraction in the trabecular bone due to difficulties of defining the border between trabecular and cortical bone. The correlation between trabecular bone volume fraction and cortical porosity was substantially weaker at the 14% site, indicating that assessment of porosity at that site provides a more accurate measurement. Furthermore, as the variation in bone length introduces a systematic error in the estimation of cortical porosity[43] and other HR-pQCT–derived bone variables, the use of a measure that relates to bone length such as the 14% site is likely to provide more accurate measurements. Based upon these issues we propose that evaluating cortical porosity is more accurate at a more proximal site measured at a distance relative to bone length. By using such a measurement we observed a lower cortical porosity at the 14% site of the radius and no difference in the tibia between T2DM and controls, arguing against higher cortical porosity in T2DM. This is also congruent with the observation of normal or higher values in other bone microarchitecture variables in T2DM. However, differences in skeletal traits between measuring sites could not be ruled out. Both the standard and 14% sites are in close vicinity of the most common location of a typical forearm fracture[44] and could therefore be of interest to measure in order to predict these fractures. One other study included a more proximal, fixed, site to evaluate cortical porosity in T2DM,[13] but was without a comparison to a relevant control group as patients with T2DM with and without fractures were compared. As expected, patients who had sustained a fracture, regardless of a concomitant diagnosis of T2DM, had higher

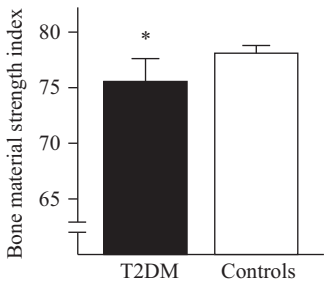

Fig. 5. BMSi measured by microindentation in subjects with (filled bars) and without (open bars) T2DM. Bars represent mean ± SE after adjustment for age, body mass index, smoking, current and previous glucocorticoid treatment, use of bone-active drugs, physical activity, and calcium intake. $^*p < 0.05$ for statistical difference between T2DM and controls. BMSi = bone material strength index.

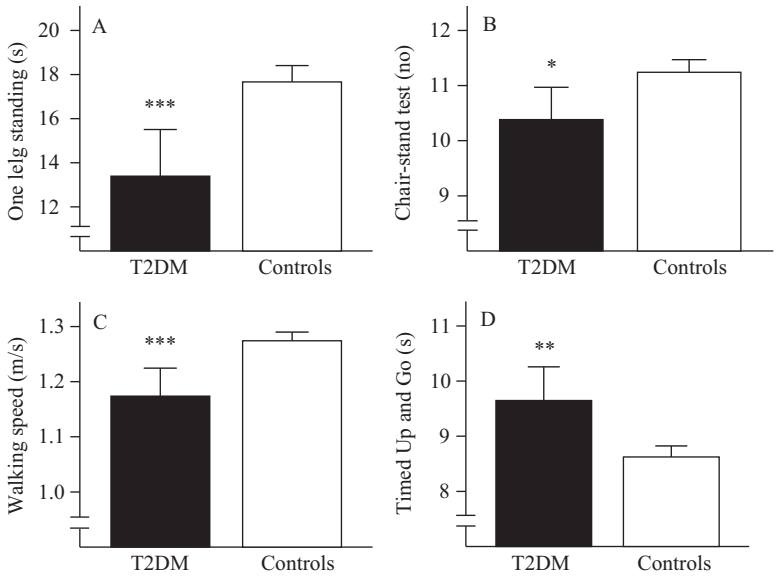

Fig. 6. Tests of physical function (one leg standing test [*A*], chair-stand test [*B*], walking speed [*C*], and timed up and go [*D*]) in subjects with (filled bars) and without (open bars) T2DM. Bars represent mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for statistical difference between T2DM and controls.

cortical porosity than subjects without fractures but there was no comparison between fracture patients with and without T2DM.[13]

In a relatively large Danish study, aBMD and bone microarchitecture were compared between type 1 diabetes mellitus (T1DM) and T2DM patients attending university clinics.[45] In comparison, subjects with T2DM had higher *T*-scores for hip, femur, and spine aBMD, higher tissue stiffness in the tibia, and higher cortical pore volume in the radius compared to T1DM. Previous studies have reported a higher risk of fractures in T1DM than in T2DM,[4] indicating that the causes for bone fragility may be partly different in these diagnoses. Hyperglycemia and hypoglycemia, accumulation of AGEs, type of pharmacological therapy, and vascular complications are all factors that may influence bone health in diabetes mellitus.[46–48] The pathogenesis for bone disease is likely to be multifactorial, especially in T2DM, and therefore difficult to elucidate in small studies, because lifestyle factors such as inactivity and obesity are well known to contribute both to the development of T2DM and to the risk of fracture.[49]

In contrast to the more favorable cortical and trabecular microstructure, subjects with T2DM performed worse in physical function tests including TUG, rising from a chair, and walking speed. These tests are relatively easy to perform and have been shown to reflect physical function of importance for the daily life in the elderly.[23–25] A slower TUG test has been reported to be an independent predictor of fracture risk in older women.[50] Previous studies have also shown that the risk of falling is increased in T2DM.[21] Poor physical function may be secondary to low physical activity that may contribute both to the development of T2DM and osteoporosis. However, current physical activity was included in our adjusted comparisons and a better bone microarchitecture was still seen in T2DM.

Bone material strength measurement in vivo by microindentation is a relatively new method. Previous studies have shown differences between fracture patients and controls, and the effects of different bone-active drugs in glucocorticoid-treated subjects.[37,51] In the latter study, there was detectable change in BMSi already after 7 weeks. BMSi is correlated to bone toughness in some[52] but not all[53] experimental studies. Previous smaller studies in subjects with T2DM have shown lower values compared to controls[12,18] and our study confirms this in a larger population-based study. However, because it is unknown how the BMSi translates to the clinical risk for fracture, the importance of this finding remains unclear.

Among the limitations of our study is that it only includes ambulant women and there is a risk that there is a selection of relatively fit T2DM patients in our study. This may be the reason for not finding a difference in fracture rate, and for the high quality of bone microarchitecture in T2DM. There was, however, a clear reduction both in BMSi and in physical function in T2DM that could indicate a higher risk of fracture. Among the strengths of our study are the high number of subjects with T2DM with detailed bone phenotype and its population-based design with a large control group. Thus, in our view, it can be used for conclusions regarding bone phenotype in ambulant women aged 75 to 80 years of age with T2DM.

In conclusion, our results confirm that areal BMD is not reduced whereas BMSi is lower in T2DM. However, trabecular and cortical microarchitecture as well as biomechanical properties by FEA was clearly better in this large group of women with T2DM compared to a population-based control group, whereas different measures of physical function were clearly worse. We therefore suggest that a significant proportion of the increased fracture risk in T2DM depends on physical impairment and perhaps on reduced bone material strength. It remains to be shown if improved physical function can reduce the risk of fractures in T2DM.

Disclosures

All authors state that they have no conflicts of interest.

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References

1. Schwartz AV, Sellmeyer DE, Ensrud KE, et al. Older women with diabetes have an increased risk of fracture: a prospective study. J Clin Endocrinol Metab. 2001;86:32–8.
2. Bonds DE, Larson JC, Schwartz AV, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. J Clin Endocrinol Metab. 2006;91:3404–10.
3. Janghorbani M, Van Dam RM, Willet WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol. 2007;166:495–505.
4. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporos Int. 2007;18:427–44.
5. Strotmeyer ES, Cauley JA, Schwartz AV, et al. Diabetes is associated independently of body composition with BMD and bone volume in older white and black men and women: The Health, Aging, and Body Composition Study. J Bone Miner Res. 2004;19:1084–91.
6. Griangregorio LM, Leslie WD, Lix LM, et al. FRAX underestimates fracture risk in patients with diabetes. J Bone Miner Res. 2012;27:301–8.
7. Farr JN, Khosla S. Determinants of bone strength and quality in diabetes mellitus in humans. Bone. 2016;82:28–34.
8. Rubin MR, Patsch JM. Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions. Bone Res. 2016;4:16001.
9. Sellmeyer DE, Civitelli R, Hofbauer LC, Khosla S, Lecka-Czernik B, Schwartz AV. Skeletal metabolism, fracture risk, and fracture outcomes in type 1 and type 2 diabetes. Diabetes. 2016;65:1757–66.
10. Consensus Development Conference. Prophylaxis and treatment of osteoporosis. Am J Med. 1991;90:107–10.
11. Shu A, Yin MT, Stein E, et al. Bone structure and turnover in type 2 diabetes mellitus. Osteoporos Int. 2012;23:635–41.
12. Farr JN, Drake MT, Amin S, Melton LJ, McCready LK, Khosla S. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. J Bone Miner Res. 2014;29:787–95.
13. Patsch JM, Burghardt AJ, Yap SP, et al. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. J Bone Miner Res. 2013;28:313–24.
14. Heilmeier U, Cheng K, Pasco C, et al. Cortical bone laminar analysis reveals increased midcortical and periosteal porosity in type 2 diabetic postmenopausal women with history of fragility fractures compared to fracture-free diabetics. Osteoporos Int. 2016 Sep;27(9):2791–802.
15. Burghardt AJ, Issever AS, Schwartz AV, et al. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2010;95:5045–55.
16. Yu EW, Putman MS, Derrico N, Abrishamanian-Garcia G, Finkelstein JS, Bouxsein ML. Defects in cortical microarchitecture among African-American women with type 2 diabetes. Osteoporosis Int. 2015;26:673–9.
17. Paccou J, Ward KA, Jameson KA, Dennison EM, Cooper C, Edwards MH. Bone microarchitecture in men and women with diabetes: the importance of cortical porosity. Calcif Tissue Int. 2016;98:465–73.
18. Furst JR, Bandeira LC, Fan W-W, et al. Advanced glycation end products and bone material strength in type 2 diabetes. J Clin Endocrinol Metab. 2016;101:2502–10.
19. Karim L, Bouxsein ML. Effect of type 2 diabetes-related non-enzymatic glycation on bone biomechanical properties. Bone. 2016;82:21–7.
20. Gregg EW, Beckles GLA, Williamson DF, et al. Diabetes and physical disability among older U.S. adults. Diabetes Care. 2000;23:1272–7.
21. Schwartz AV, Hillier TA, Sellmeyer DE, et al. Older women with diabetes have a higher risk of falls: a prospective study. Diabetes Care. 2002;25:1749–54.
22. Vestergaard P, Rejnmark L, Mosekilde L. Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes. Calcif Tissue Int. 2009;84:45–55.
23. Podsiado D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc. 1991;39:142–8.
24. Jones CJ, Rikli RE, Beam WC. A 30-s chair-stand test as a measure of lower body strength in community-residing older adults. Res Q Exerc Sport. 1999;70:113–9.
25. Giorgetti MM, Harris BA, Jette A. Reliability of clinical balance outcome measures in the elderly. Physiother Res Int. 1998;3:274–83.
26. Washburn RA, McAuley E, Katula J, Mihalko SL, Boileau RA. The physical activity scale for the elderly (PASE): evidence for validity. J Clin Epidemiol. 1999;52:643–51.
27. Angbratt M, Möller M. Questionnaire about calcium intake: can we trust the answers? Osteoporos Int. 1999;9:220–5.
28. MacNeil JA, Boyd SK. Improved reproducibility of high-resolution peripheral quantitative computed tomography for measurement of bone quality. Med Eng Phys. 2008;30:792–9.
29. Sundh D, Rudäng R, Zoulakis M, Nilsson AG, Darelid A, Lorentzon M. A high amount of local adipose tissue is associated with high cortical porosity and low bone material strength in older women. J Bone Miner Res. 2016;31:749–57.
30. Laib A, Hauselmann HJ, Ruegsegger P. In vivo high resolution 3D-QCT of the human forearm. Technol Health Care. 1998;6:329–37.
31. Hildebrand T, Rüegsegger P. A new method for the model-independent assessment of thickness in three-dimensional images. J Microsc. 1997;185:67–75.
32. Parfitt AM, Mathews CHE, Villanueva AR, Kleerekoper M, Frame B, Rao DS. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest. 1983;72:1396–409.
33. Burghardt AJ, Buie HR, Laib A, Majumdar S, Boyd SK. Reproducibility of direct quantitative measures of cortical bone microarchitecture of the distal radius and tibia by HR-pQCT. Bone. 2010;47:519–28.
34. Ostertag A, Peyrin F, Fernandez S, Laredo JD, de Vernejoul MC, Chappard C. Cortical measurements of the tibia from high resolution peripheral quantitative computed tomography images: a comparison with synchrotron radiation micro-computed tomography. Bone. 2014;63:7–14.
35. Pistoia W, van Rietbergen B, Lochmüller EM, Lill CA, Eckstein F, Rüegsegger P. Estimation of distal radius failure load with micro-finite element analysis models based on three-dimensional peripheral quantitative computed tomography images. Bone. 2002;30:842–8.
36. Chiu J, Robinovitch SN. Prediction of upper extremity impact forces during falls on the outstretched hand. J Biomech. 1998;31:1169–76.
37. Diez-Perez A, Güerri R, Nogues X, et al. Microindentation for in vivo measurement of bone tissue mechanical properties in humans. J Bone Miner Res. 2010;25:1877–85.
38. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993;8:1137–48.

39. Rudäng R, Zoulakis M, Sundh D, et al. Bone material strength is associated with areal BMD but not with prevalent fractures in older women. Osteoporos Int. 2016;27:1585–92.
40. Sundh D, Mellström D, Nilsson M, Karlsson M, Ohlsson C, Lorentzon M. Increased cortical porosity in older men with fracture. J Bone Miner Res. 2015;30:1692–700.
41. Yeni YN, Brown CU, Wang Z, Norman TL. The influence of bone morphology on fracture toughness of the human femur and tibia. Bone. 1997;21:453–9.
42. Zebaze R, Ghasem-Zadeh A, Mbala A, Seeman E. A new method of segmentation of compact-appearing, transitional and trabecular compartments and quantification of cortical porosity from high resolution peripheral quantitative computed tomographic images. Bone. 2013;54:8–20.
43. Shanbhogue VV, Hansen S, Halekoh U, Brixen K. Use of relative vs fixed offset distance to define region of interest at the distal radius and tibia in high-resolution peripheral quantitative computed tomography. J Clin Densitom. 2015;18:217–25.
44. Meena S, Sharma P, Sambharia AK, Dawar A. Fractures of distal radius: an overview. J Family Med Prim Care. 2014;3:325–32.
45. Starup-Linde J, Lykkeboe S, Gregersen S, et al. Bone structure and predictors of fracture in type 1 and type 2 diabetes. J Clin Endocrinol Metab. 2016;101:928–36.
46. Shanbhougue VV, Hansen S, Frost M, et al. Compromised cortical bone compartment in type 2 diabetes mellitus patients with microvascular disease. Eur J Endocrinol. 2016;174:115–24.
47. Ivers RQ, Cumming RG, Mitchell P, Peduto AJ. Diabetes and risk of fracture: The Blue Mountains Eye Study. Diabetes Care. 2001;24:1198–203.
48. Nelson DA, Jacober SJ. Why do older women with diabetes have an increased fracture risk? J Clin Endocrinol Metab. 2001;86:29–31.
49. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. Compr Physiol. 2012;2:1143–211.
50. Zhu K, Devine A, Lewis JR, Dhaliwal SS, Prince RL. "Timed up and go" test and bone mineral density measurement for fracture prediction. Arch Intern Med. 2011;171:1655–61.
51. Mellibovsky L, Prieto-Alhambra D, Mellibovsky F, et al. Bone tissue properties measurement by reference point indentation in gluco-corticoid-induced osteoporosis. J Bone Miner Res. 2015;30:1651–6.
52. Gallant MA, Brown DM, Organ JM, Allen MR, Burr DB. Reference-point indentation correlates with bone toughness assessed using whole-bone traditional mechanical testing. Bone. 2013;53:301–5.
53. Carriero A, Bruse JL, Oldknow KJ, Millán JL, Farquharson C, Shefelbine SJ. Reference point indentation is not indicative of whole mouse bone measures of stress intensity fracture toughness. Bone. 2014;69:174–9.